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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte MIMI ADACHI, KEIICHI NAKAYAMA,
SHIGETAKA KITAJIMA and HIROMITSU TAKAGI

Appeal 2010-007915
Application 10/580,248
Technology Center 1600

Before TONI R. SCHEINER, DONALD E. ADAMS, and JEFFREY N. FREDMAN, *Administrative Patent Judges*.

ADAMS, *Administrative Patent Judge*.

DECISION ON APPEAL

This appeal under 35 U.S.C. § 134 involves claims 1, 4-12, 15-25, 31, 34, and 35 (App. Br. 1). Pending "[c]laims 3, 13, 14, and 26-30 stand withdrawn" from consideration (*id.*). We have jurisdiction under 35 U.S.C. § 6(b).

STATEMENT OF THE CASE

The claims are directed to methods for proliferating cardiomyocytes (claims 1, 4-12, 15, 16, 34, and 35), a vector (claims 17-25), and a cardiomyocyte (claim 31). Claims 1 and 17 are representative and are

reproduced in the "CLAIMS APPENDIX" of Appellants' Brief (App. Br. 17 and 19).

Claims 1, 4-12, 15-25, and 31 stand rejected under 35 U.S.C. § 103(a) as unpatentable over the combination of Tamamori-Adachi,[1] Sutterlüty,[2] Sherr,[3] Flink,[4] and Poolman.[5]

Claims 34 and 35 stand rejected under 35 U.S.C. § 103(a) as unpatentable over the combination of Tamamori-Adachi, Sutterlüty, Sherr, Flink, Poolman, and Carrano.[6]

We reverse the rejection of claims 17-25 and affirm all other rejections of record.

The combination of Tamamori-Adachi, Sutterlüty, Sherr, Flink, and Poolman:

ISSUE

Does the preponderance of evidence on this record support a conclusion of obviousness?

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[1] Mimi Tamamori-Adachi, et al., *Critical Role of Cyclin D1 Nuclear Import in Cardiomyocyte Proliferation*, 92 Circ. Res. e12-e19 (2003).
[2] Hedwig Sutterlüty, et al., *p45^{SKP2} promotes p27^{Kip1} degradation and induces S phase in quiescent cells*, 1 Nature Cell Biol. 207- 214 (1999).
[3] Charles J. Sherr, et al., *CDK inhibitors: positive and negative regulators of G_1-phase progression*, 13 Genes Dev. 1501-1512 (1999).
[4] Irwin L. Flink, et al., *Changes in E2F Complexes Containing Retinoblastoma Protein Family Members and Increased Cyclin-dependent Kinase Inhibitor Activities During Terminal Differentiation of Cardiomyocytes*, 30 J. Mol. Cell Cardiol. 563-578 (1998).
[5] Robert A. Poolman, et al., *Altered Expression of Cell Cycle Proteins and Prolonged Duration of Cardiac Myocyte Hyperplasia in p27^{KIP1} Knockout Mice*, 85 Circ Res. 117-127 (1999).
[6] Andrea C. Carrano, et al., *SKP2 is required for ubiquitin-mediated degradation of the CDK inhibitor p27*, 1 Nature Cell Biol. 193-199 (1999).

FACTUAL FINDINGS

FF 1. "Cell cycle progression is known to be regulated both positively, through the formation of cyclin and cyclin-dependent kinase (CDK) complexes, and negatively, by cyclin-dependent kinase inhibitors (CKDIs), that bind to and inhibit the activities of various cyclin-CDK complexes" (Poolman 117: col. 1, ll. 14-18).

FF 2. Tamamori-Adachi teaches "that postmitotic cardiomyocytes have the potential to proliferate provided that cyclin D1/CDK4 accumulate in the nucleus, and the prevention of their nuclear import plays a critical role as a physical barrier to prevent cardiomyocyte proliferation" (Ans. 4).

FF 3. Similarly, Flink teaches that "cardiomyocytes retain the capacity to proliferate until the early neonatal period when a series of changes occur, including . . . a decrease in CDK levels and induction of [p27] CDK inhibitory activity, which is associated with terminal differentiation" (Flink, 563: Abstract; *see also* Ans. 6 (Flink "teach[es] that during terminal differentiation of cardiomyocytes p27 is increased")).

FF 4. Sherr teaches that "CDK inhibitors such as $p27^{Kip1}$ negatively regulate cell cycle progression" (Ans. 5; *see also* Poolman 125: col. 1, ll. 41-42 ("$p27^{KIP1}$ is part of the mechanism that determines when cells stop dividing and differentiate")).

FF 5. Flink teaches that "[a]lthough the mRNA levels of the CDK inhibitor, p27, were not changed in neonatal cells, its protein and inhibitory activity . . . were increased" (Flink 564: col. 2, ll. 53-55).

FF 6. Sutterlüty teaches that "$p27^{Kip1}$ levels are high in quiescent cells but fall once cells enter the cell cycle" (Sutterlüty 207: col. 1, ll. 39-40).

FF 7. Tamamori-Adachi teaches that the nuclear localization and co-expression of cyclin D1 and cyclin-dependent kinase 4 (CDK4), using adenovirus constructs containing cyclin D1 and cyclin-dependent kinase 4, promoted the proliferation of rat neonatal cardiomyocytes in culture (Ans. 3 and 11).

FF 8. Tamamori-Adachi does not teach "the introduction of a gene encoding a factor that inhibits the production or function of Cip/[K]ip family proteins into cardiomyocyte cultures" (Ans. 4).

FF 9. Sutterlüty teaches that the elimination of $p27^{Kip1}$ is required for cells to transition from a quiescent state to a proliferative state (Sutterlüty 207: col. 1, ll. 36-38).

FF 10. Sutterlüty teaches that $p45^{SKP2}$ promotes $p27^{Kip1}$ degradation and induces S phase in quiescent cells (Ans. 4).

FF 11. Poolman teaches that, in mice, knocking out the expression of $p27^{Kip1}$ "resulted in a significant increase in heart size and in the total number of cardiac myocytes" (Ans. 6).

FF 12. For clarity, we reproduce Fig. 8 of Appellants' Specification below:

Fig. 8

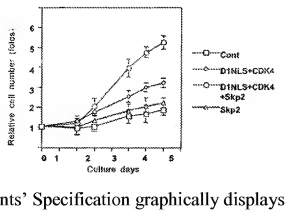

Figure 8 of Appellants' Specification graphically displays the results of a study of the "[e]ffect of enforced Skp2 gene expression on the promotion of the proliferation of cardiomyocytes" (Spec. 26: 12-13).

FF 13. Appellants disclose that

> [T]he cell number of the cardiomyocytes with D1NLS and CDK4 genes expressed therein was increased about 3 fold on day 7 post-culturing (Fig. 8). It was alternatively confirmed that the cell number of cardiomyocytes with the three genes namely D1NLS, CDK4 and Skp2 genes expressed therein was increased 5 fold or more. Almost no increase of the cell numbers of cardiomyocytes infected with control vector and cardiomyocytes infected with Ad-Skp2 alone as negative controls was observed.

(Spec. 78: 8-16.)

ANALYSIS

Based on the combined teachings of Tamamori-Adachi, Sutterlüty, Sherr, Flink, and Poolman the Examiner concludes that, at the time of Appellants' invention, it would have been prima facie obvious to induce in vitro cultured cardiomyocytes to enter a proliferative state by co-transfecting a cardiomyocyte with an expression construct comprising Sutterlüty's $p45^{SKP2}$ along with Tamamori-Adachi's cyclin D1 and CDK4 constructs (Ans. 7). We find no error in the Examiner's prima facie case of obviousness.

Claim 1:

Each of Sutterlüty, Sherr, Flink, and Poolman suggest that $p27^{Kip1}$ exerts a negative influence on cell-cycle progression (*see* FF 3-6 and 9-11). Sutterlüty and Poolman expressly teach that the removal of $p27^{Kip1}$, a negative regulator of cell cycle progression, allowed the cells to enter the S phase of the cell-cycle (FF 4 and 9-11). Accordingly, we are not persuaded by Appellants' contention that the combined teachings of Tamamori-Adachi,

Sutterlüty, Sherr, Flink, and Poolman fail to suggest element (c) of claim 1 (App. Br. 6).

We are not persuaded by Appellants' contention that Sutterlüty "is unrelated to the claimed invention" because it addresses cell cycle proteins such as cyclin-D, CDK4, and $P27^{Kip1}$ in the context of quiescent fibroblasts, not cardiomyocytes (App. Br. 7; *Cf.* FF 7 and 11). The preponderance of evidence on this record establishes that the cell cycle regulatory proteins cyclin-D, CDK4, and $p27^{Kip1}$ are involved in regulating the cell cycle of cardiomyocytes (*see* FF 2, 7, and 11). There is no persuasive evidence or argument on this record to support a conclusion that the observations made by Sutterlüty regarding cell cycle regulation of quiescent fibroblasts are not equally applicable to cell cycle regulation of cardiomyocytes. The same is true for Appellants' contentions regarding Sherr's discussion of quiescent cells (App. Br. 8).

Flink addresses the involvement of $p27^{Kip1}$ and other cell cycle proteins in cardiomyocyte cell cycle regulation (FF 3 and 5). Accordingly, we are not persuaded by Appellants' contentions, which address Flink in isolation as opposed to Flink's teachings in combination with Tamamori-Adachi, Sutterlüty, Sherr, and Poolman (App. Br. 8). The same is true of Appellants' contentions regarding the teachings of Poolman in isolation (*see* App. Br. 9).

We disagree with Appellants' contention that Appellants' Specification teaches that the introduction of $p45^{SKP2}$ into cardiomyocytes "***does not*** result in an increase in the proliferation of cardiomyocytes" (App. Br. 10; *see also* Reply Br. 6-7). Appellants' Specification discloses that "[a]lmost no increase" in proliferation was seen with cells transfected with

Ad-Skp2 (FF 13; *see also* App. Br. 10-11 (""almost no increase of the cell numbers of cardiomyocytes infected [with] Skp2 gene alone was observed")). The results illustrated in Appellants' Fig. 8 provide evidence of a 2 fold increase in cardiomyocyte proliferation when cells are transfected with Ad-Skp2 (FF 12). Fig. 8 also corresponds to Appellants' disclosure that a 3 fold increase was observed when cardiomyocytes were co-transfected with cyclin-D (D1NLS) and CDK4; and a 5 fold increase was observed when the cells were co-transfected with D1NLS, CDK4, and Skp2 (FF 13). Taken as a whole, the data presented in Appellants' Specification supports a conclusion that the increase in proliferation was additive. The preponderance of evidence on this record supports a conclusion that such an additive increase in proliferation would have been expected by those of ordinary skill in this art, wherein cell cycle progression is induced by transfecting a cardiomyocyte with cyclin-D and CDK; and the negative cell-cycle inhibitor $p27^{Kip1}$ is removed by transfecting the same cardiomyocyte with $p45^{SKP2}$, or other means to inhibit the production, function, or action of a Cip/Kip family protein (FF 1-11). Accordingly, we are not persuaded by Appellants' contention that their Specification supports a conclusion of unexpected results (App. Br. 10-12 and 14; Reply Br. 6-7).

Flink teaches that "[a]lthough the mRNA levels of the CDK inhibitor, p27, were not changed in neonatal cells, its protein and inhibitory activity . . . were increased" (FF 5). Accordingly, we are not persuaded by Appellants' contentions that "expression of $p27^{Kip1}$ siRNA alone does not result in an increase in the number of cardiomyocytes" (emphasis omitted (App. Br. 11; Reply Br. 6-7)). Appellants provide no persuasive evidence or reasoning to support a conclusion that the expression of $p27^{Kip1}$ siRNA *alone* in a

quiescent cardiomyocyte affects the concentration of the pre-existing $p27^{Kip1}$ inhibitory *protein* in the cell.

Notwithstanding Appellants' contentions to the contrary, the results obtained by Appellants' are suggested by the prior art. Since $p27^{Kip1}$ levels are regulated at the level of translation, not transcription (FF 5), and "$p27^{Kip1}$ levels . . . fall once cells enter the cell cycle" (FF 6); a person of ordinary skill in this art would *not* have expected a construct which inhibits $p27^{Kip1}$ transcription to have an effect on cell cycle progression, until an overabundance of cyclin-D and CDK4, such as that resulting from the introduction of Tamamori-Adachi's adenovirus constructs into the cells, "kickstarts" the proliferative cycle. Once the cell enters a proliferative cycle, $p27^{Kip1}$ protein levels drop and then a construct that prevents the production of more $p27^{Kip1}$, such as Appellants' siRNA construct, would have been expected to prevent $p27^{Kip1}$ from negatively affecting proliferation by inhibiting the accumulation of $p27^{Kip1}$ protein. Accordingly, we are not persuaded by Appellants' contentions regarding unexpected results (*see* App. Br. 14; Reply Br. 6-7). Further, while Appellants' Brief contends that the results are "surprising and unexpected", Appellants fail to direct our attention to a portion of their Specification that characterizes the results as "surprising and unexpected" (*see id.*).

Claim 17:

Appellants contend that the combination of references relied upon by the Examiner fail to suggest a single vector comprising cyclin D1, CDK4, and a gene encoding a factor that inhibits the production, function or action of a Cip/Kip family protein (App. Br. 12).

The Examiner does not address the requirements of claim 17 or Appellants' contentions regarding claim 17. Accordingly, we are compelled to reverse the Examiner's rejection as it relates to claim 17 and 18-25, which depend directly or indirectly on claim 17.

CONCLUSION OF LAW

The preponderance of evidence on this record supports a conclusion of obviousness with respect to claim 1. The rejection of claim 1 under 35 U.S.C. § 103(a) as unpatentable over the combination of Tamamori-Adachi, Sutterlüty, Sherr, Flink, and Poolman is affirmed. Because they are not separately argued claims fall together with claims 4-12, 15, 16, and 31 fall together with claim 1; and claims 18-25 fall together with claim 17. 37 C.F.R. § 41.37(c)(1)(vii).

The preponderance of evidence on this record fails support a conclusion of obviousness with respect to claims 17-25. The rejection of claims 17-25 under 35 U.S.C § 103(a) as unpatentable over the combination of Tamamori-Adachi, Sutterlüty, Sherr, Flink, and Poolman is reversed.

The combination of Tamamori-Adachi, Sutterlüty, Sherr, Flink, Poolman, and Carrano:

ISSUE

Does the preponderance of evidence on this record support a conclusion of obviousness?

FACTUAL FINDINGS

FF 14. The Examiner relies on the combination of Tamamori-Adachi, Sutterlüty, Sherr, Flink, and Poolman as discussed above (Ans. 8).

FF 15. The Examiner relies on Carrano to teach that "$p27^{Kip1}$ degradation is accomplished via SKP2 ubiquitination" (*id.*).

ANALYSIS

Appellants contend that Carrano fails to remedy the deficiencies in the combination of Tamamori-Adachi, Sutterlüty, Sherr, Flink, and Poolman (App. Br. 15). Having found no deficiency in the combination of Tamamori-Adachi, Sutterlüty, Sherr, Flink, and Poolman we are not persuaded by Appellants' contention to the contrary.

CONCLUSION OF LAW

The preponderance of evidence on this record supports a conclusion of obviousness. The rejection of claim 34 under 35 U.S.C. § 103(a) as unpatentable over the combination of Tamamori-Adachi, Sutterlüty, Sherr, Flink, Poolman, and Carrano is affirmed. Because it is not separately argued claim 35 falls together with claim 34. 37 C.F.R. § 41.37(c)(1)(vii).

TIME PERIOD FOR RESPONSE

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a).

AFFIRMED-IN-PART

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